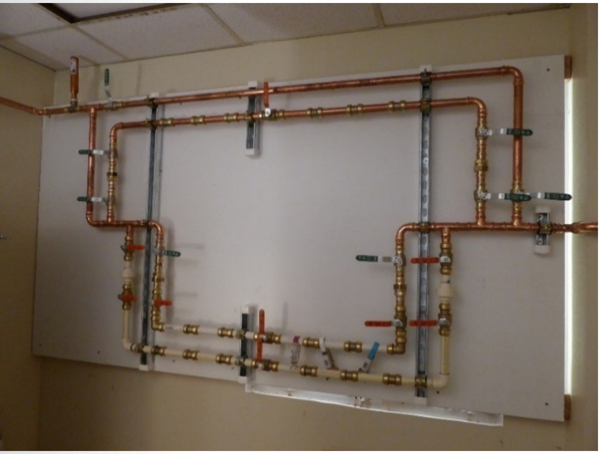
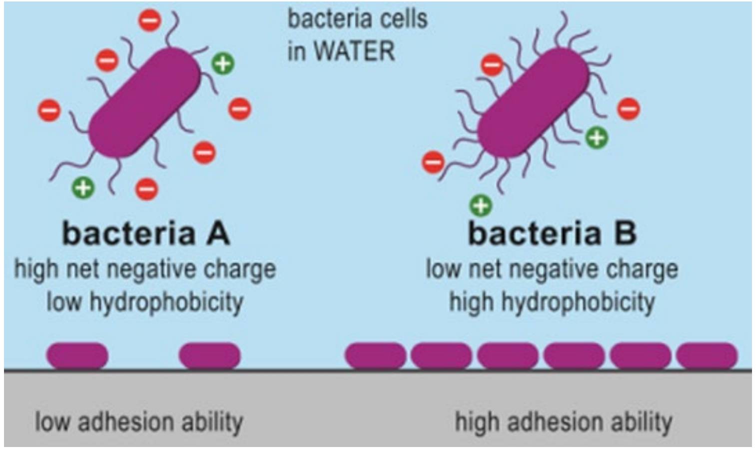
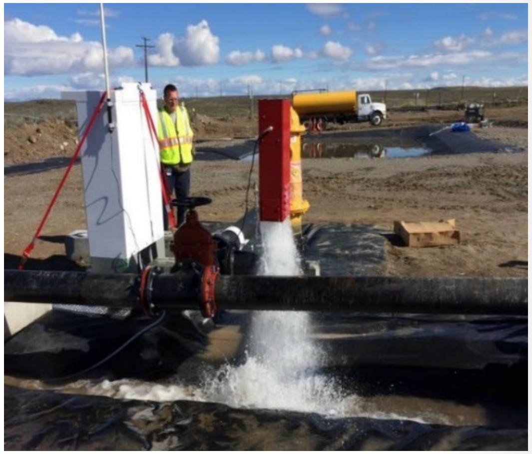
USEPA Office of Research and Development

Homeland Security Research Program







Premise Plumbing Research in EPA's Homeland Security Research Program

Jeff Szabo, Helen Buse, John Hall and Jim Goodrich

December 11, 2018

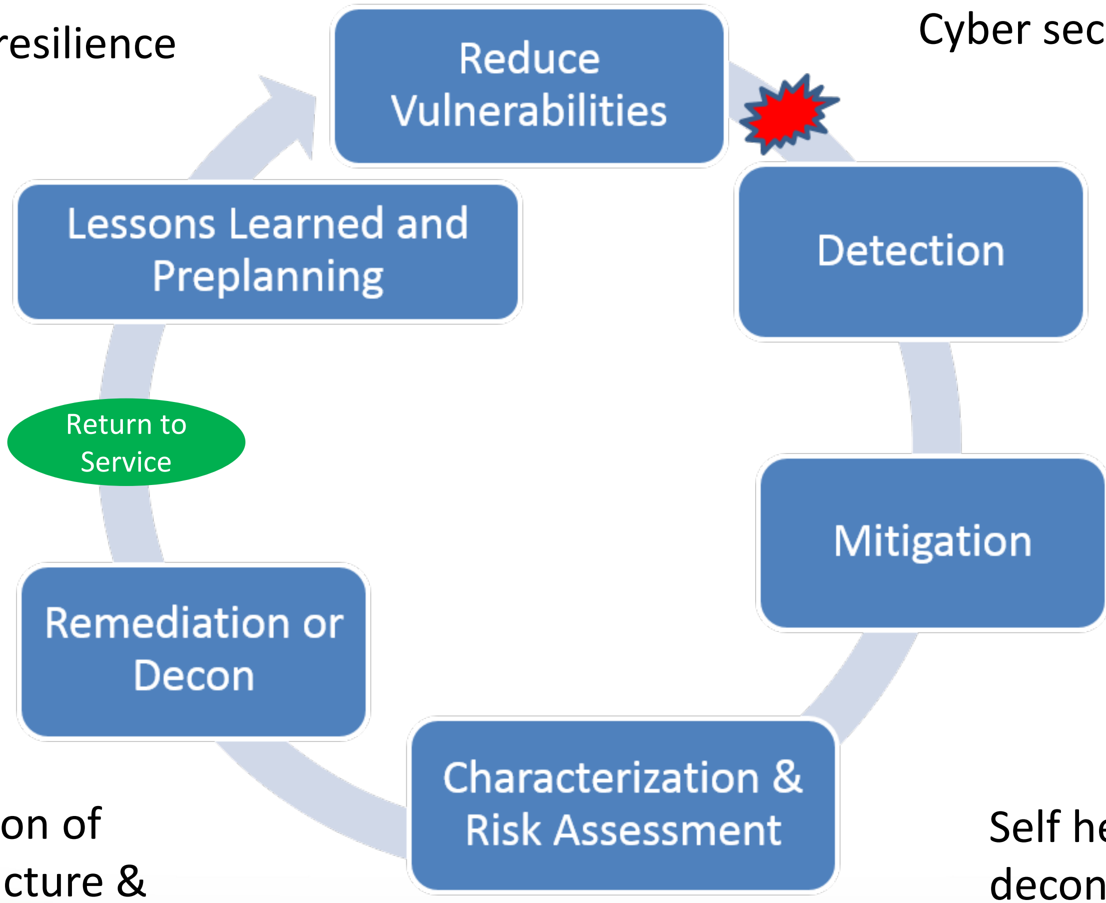
Presentation Overview

- Brief overview of the Homeland Security Research Program (HSRP)
- How the HSRP conducts premise plumbing research
- Bench scale research
  - *Legionella*
- Field or full scale research
  - Chemical and microbial contaminants

Program Design: A Systems Approach to Incidents

Increasing resilience

Cyber security for water systems



On-line monitoring of contaminants in drinking water systems

Contaminant fate and transport

Self help decontamination and risk reduction measures

Sampling and analysis for water and waste water

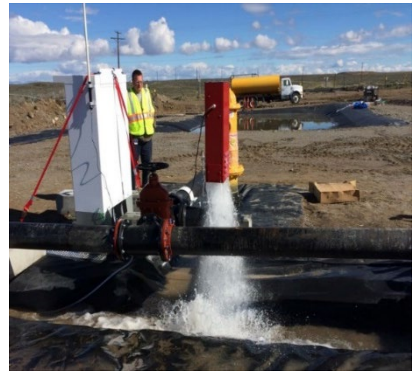
Decontamination of water infrastructure & water treatment

Wastewater treatment

Applied Research Solutions Approach

Bench-Scale

Pilot-Scale



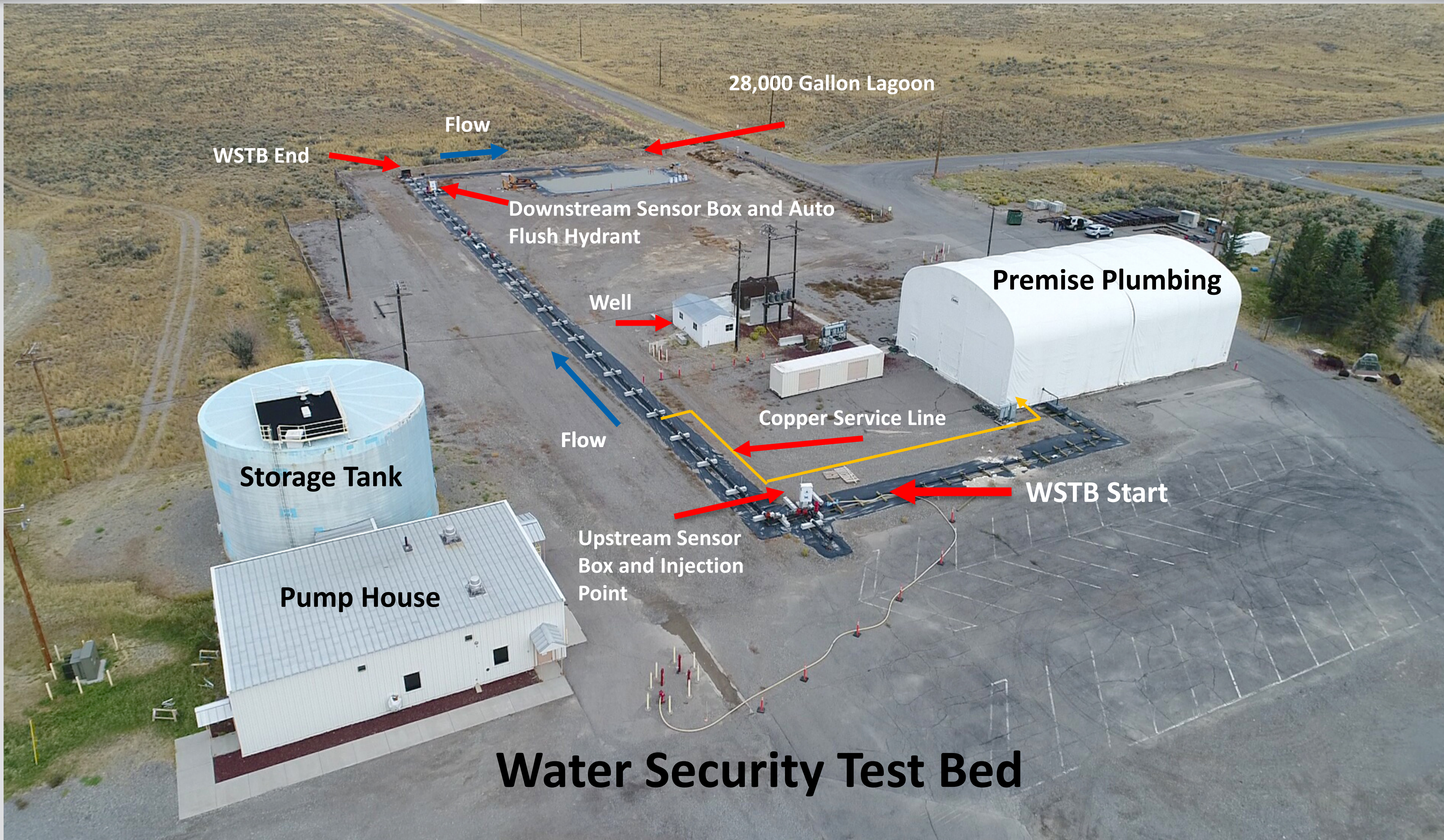
Full-Scale

Application to Real Response Incidents

www.epa.gov/homeland-security-research

Water Security Test Bed

[Water Security Test Bed Video: https://www.youtube.com/watch?v=pQvsBC-U4a8](https://www.youtube.com/watch?v=pQvsBC-U4a8)



Triggered Flushing and Online Sensors

Removable Coupons and Pipe Available for Decontamination Experiments

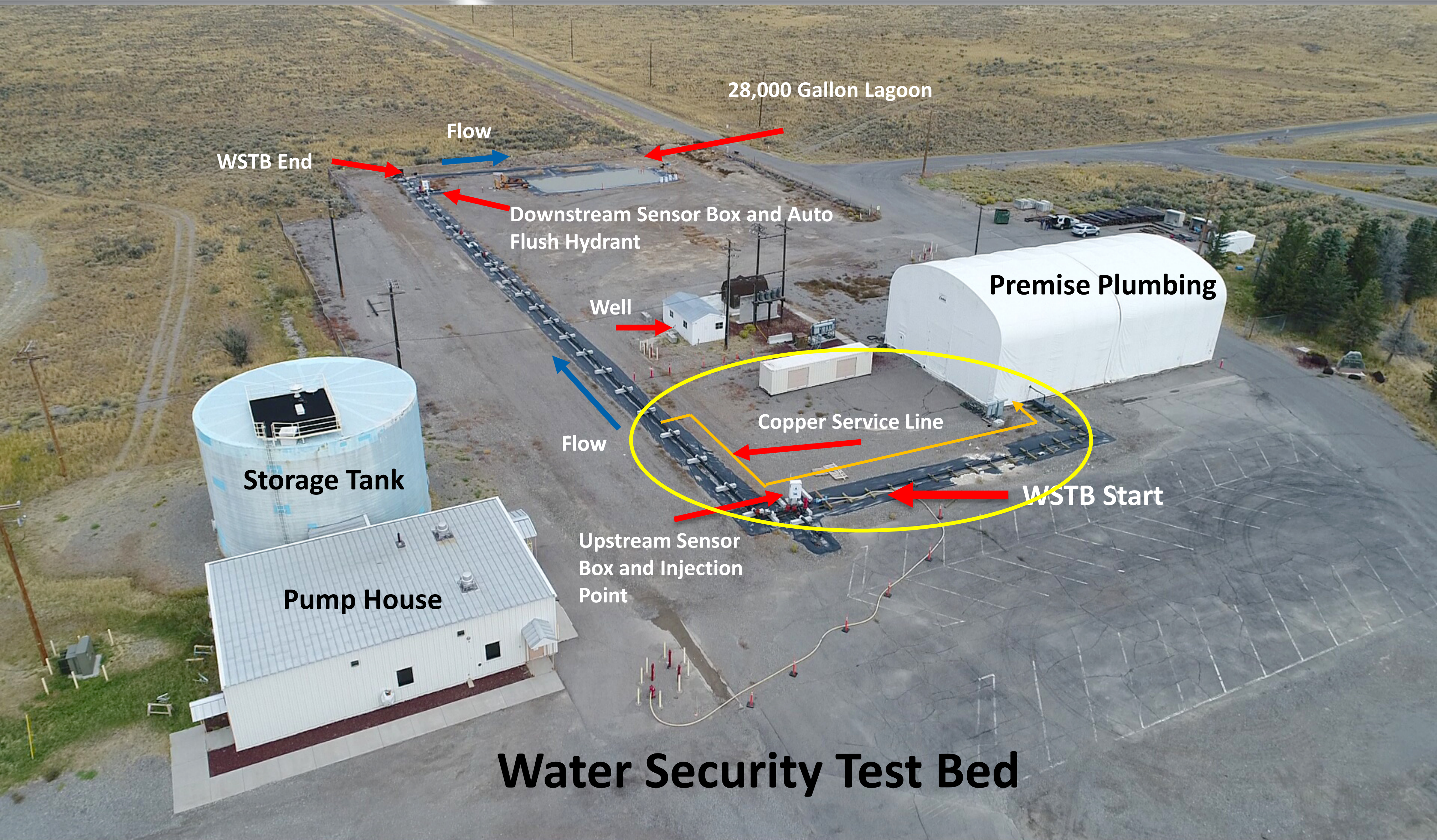
Forty year old conveyance pipes (cement mortar lined ductile iron) servicing a decommissioned building was dug out of the ground at INL

8” ductile iron

28,000 Gallon Lagoon, Tanker Truck and Treatment System

Water Security Test Bed Capability

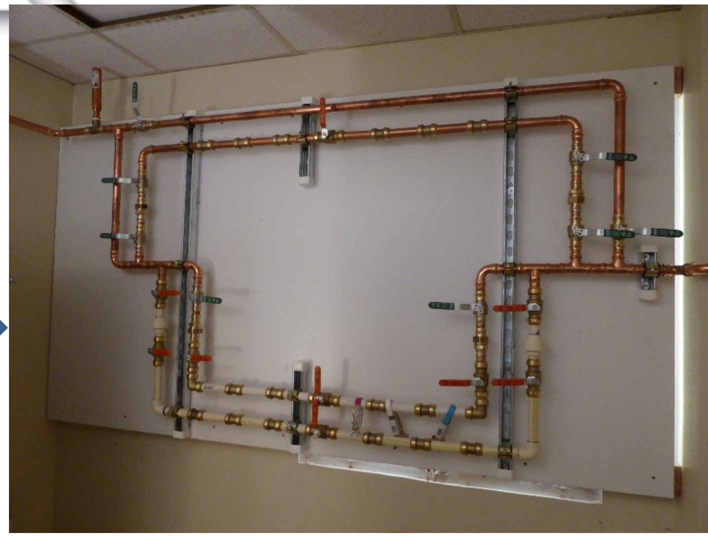
Water Security Test Bed Video: https://www.youtube.com/watch?v=pQvsBC-U4a8



Service Connection Overview

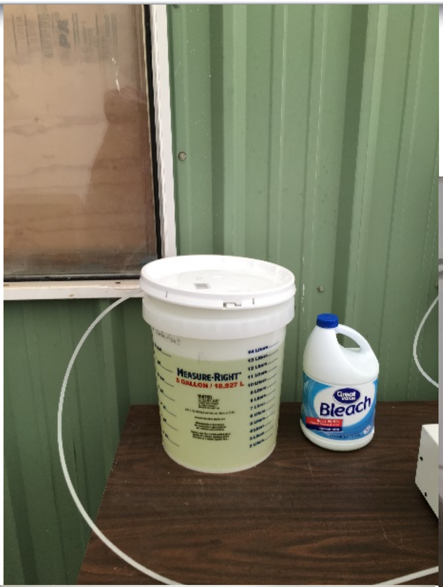
1" Copper Service Line to Indoor Plumbing (~ 200')

Premise Plumbing Setup



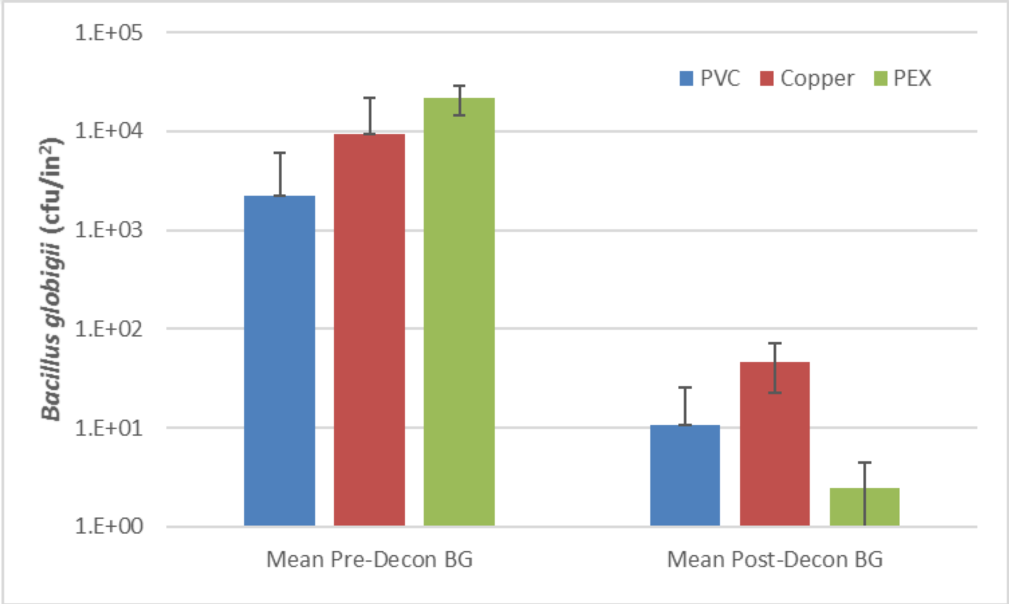
Plumbing System Spore Decontamination

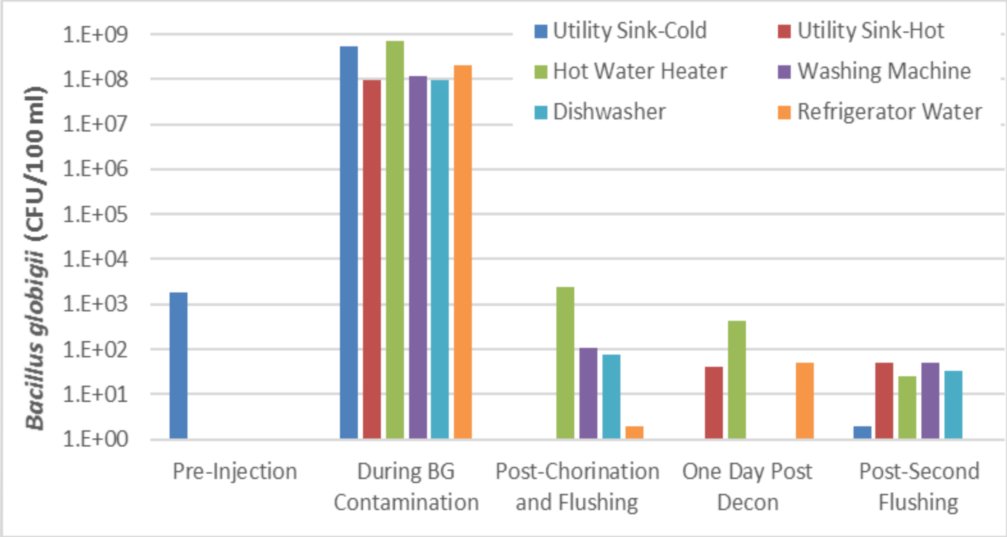
- WSTB plumbing system was contaminated with *Bacillus globigii* (BG) spores
  - BG injected at 10^6 cfu/ml in the bulk water phase
  - Injection occurred for 1 hr
- Disinfection and Flushing:
  - Amended bleach added to plumbing and allowed to sit for 1 hour
  - Cold water and refrigerator flushed for 20 min (hot water off)
  - Hot water heater drained, refilled, then hot water flushed for 75 min
  - The flushing process was repeated the next day



Plumbing Microbial Decontamination

- Decontamination with flushing and amended bleach
  - 2,300 mg/L in the plumbing and appliances, 660 mg/L in the hot water heater
  - System then flushed: cold water for 20 min, hot water for 75 min (plus draining/refilling the hot water heater)
- 2-3 log reduction of spores on pipe materials
- 6-8 log reduction from flushing the water phase
  - More persistence in the appliances
- More flushing may be needed



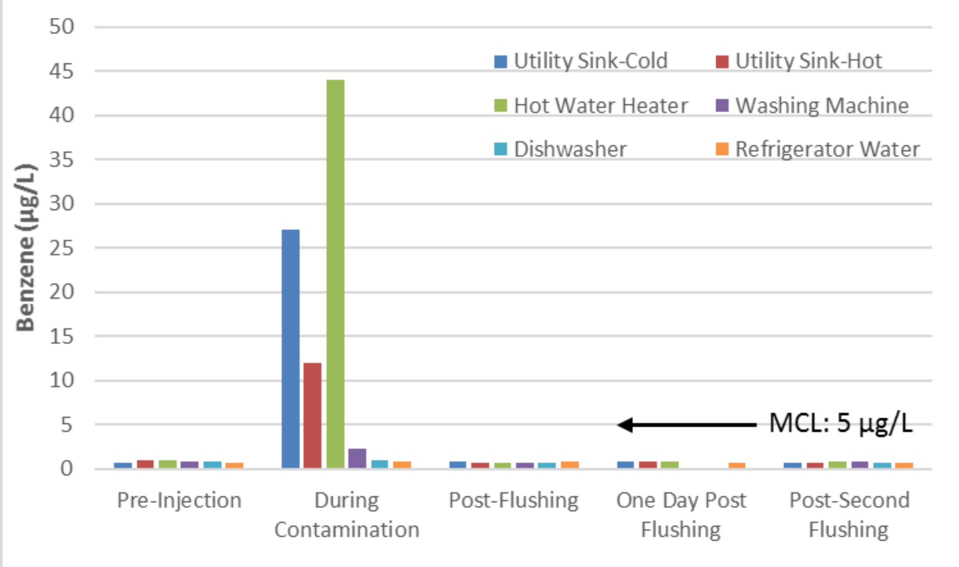


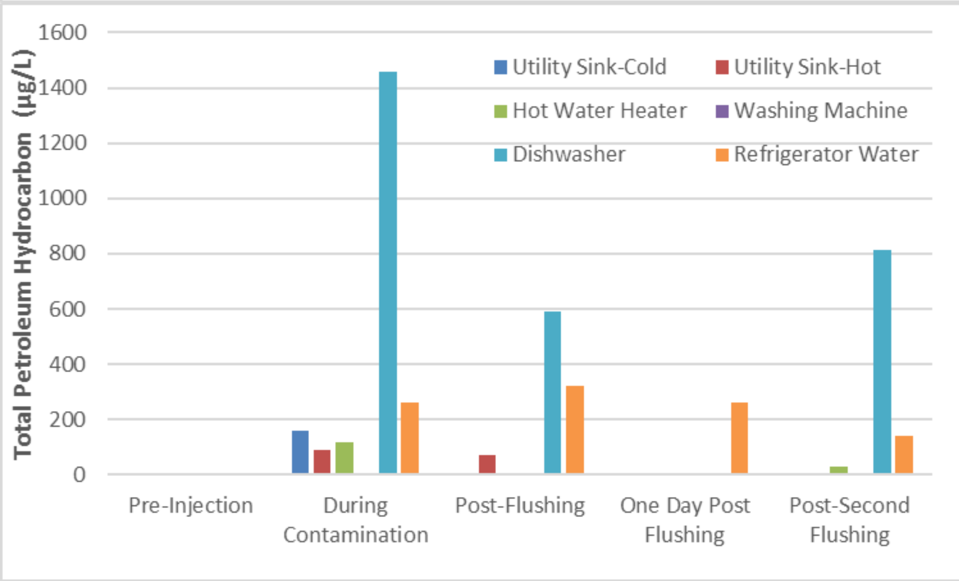
Bakken Oil Decontamination

- Injected the "subnatant" water layer from the mixture of oil and water
- One hour injection and flushing water through the premise plumbing and appliances
- Decontamination:
    - Flushing with cold water for 20 min
    - Drain water heater, refill and flush hot water plumbing for 75 min
    - Run appliances for one cycle
    - Next day, sampled and redid the decon procedure again

Bakken Oil Flushing Data

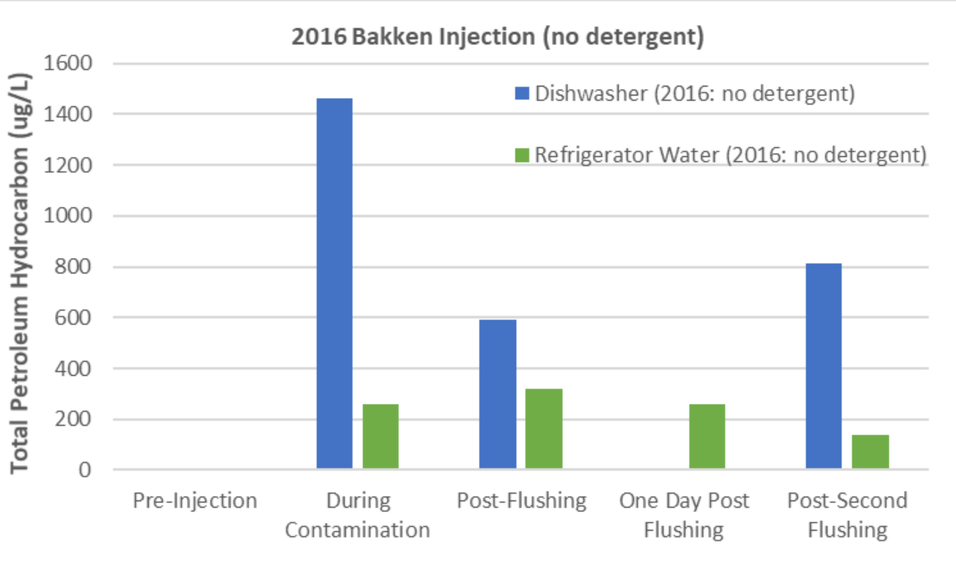
- Flushing was effective at removing BTEX
  - Benzene results shown
  - Similar results for toluene, ethylbenzene and xylenes
- Total petroleum hydrocarbon (TPH) represents a broad range of organics in oil
  - Elevated TPH observed in the refrigerator water dispenser and dishwasher after flushing

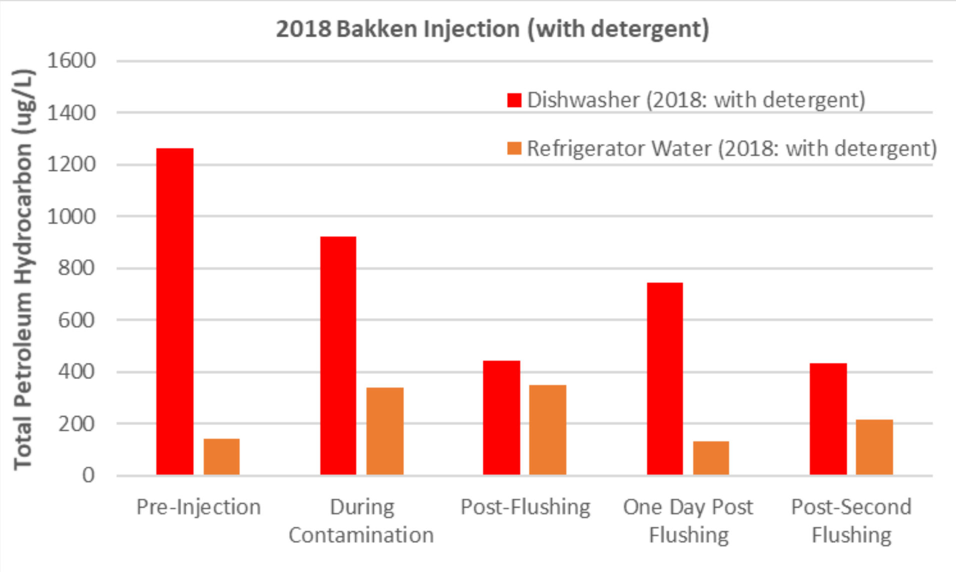




Bakken Oil Flushing with Detergents

- Flushing conducted the same as before, with detergents:
  - Cold water and refrigerator flushed for 20 min with detergent (hot water off)
  - Hot water heater drained, refilled, then hot water flushed for 75 min, appliances run
  - The flushing process was repeated the next day
  - Dawn dishwashing liquid added to refrigerator water dispenser
  - Dishwasher operated with a Cascade dishwasher pod



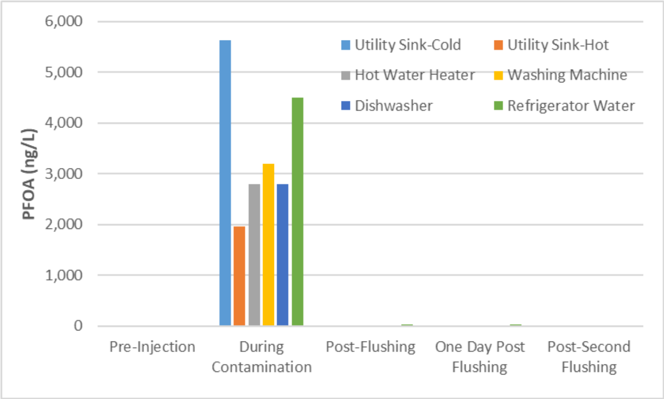


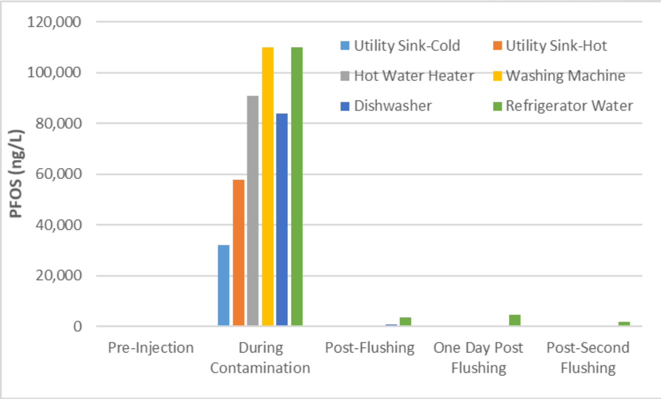
Aqueous Film Forming Foam (AFFF) Decontamination

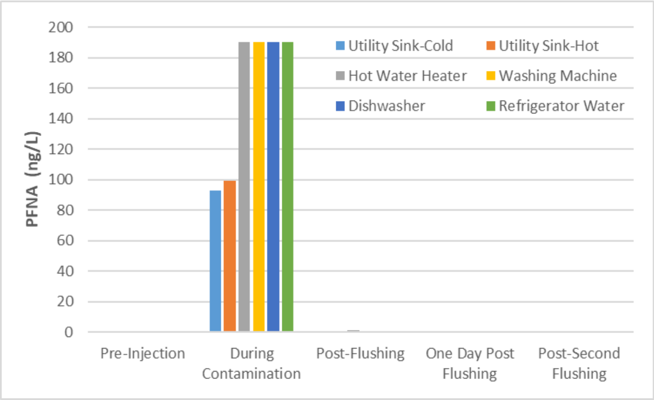
- Injected the "subnatant" water layer from the mixture of oil and water with AFFF
- One hour injection and flushing water through the premise plumbing and appliances
- Decontamination:
  - Flushing with cold water for 20 min
  - Drain water heater, refill and flush hot water plumbing for 75 min
  - Run appliances for one cycle
  - Next day, sampled and redid the decon procedure again

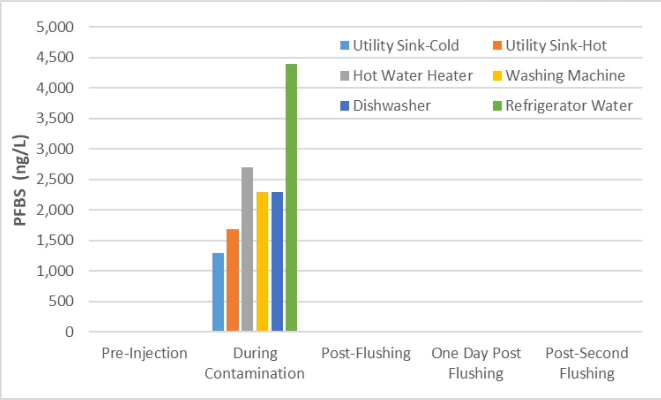
AFFF Flushing Data

- Analyzed for a variety of Per- and polyfluoroalkyl substances (PFAS)
  - PFOS, FPOA, PFBS, PFNA, etc.
- Flushing was effective at removing PFAS
  - Most PFAS compounds were not detectable after flushing (99.9+%)
  - PFOS and PFOA were detected at elevated levels in the refrigerator water and dishwasher

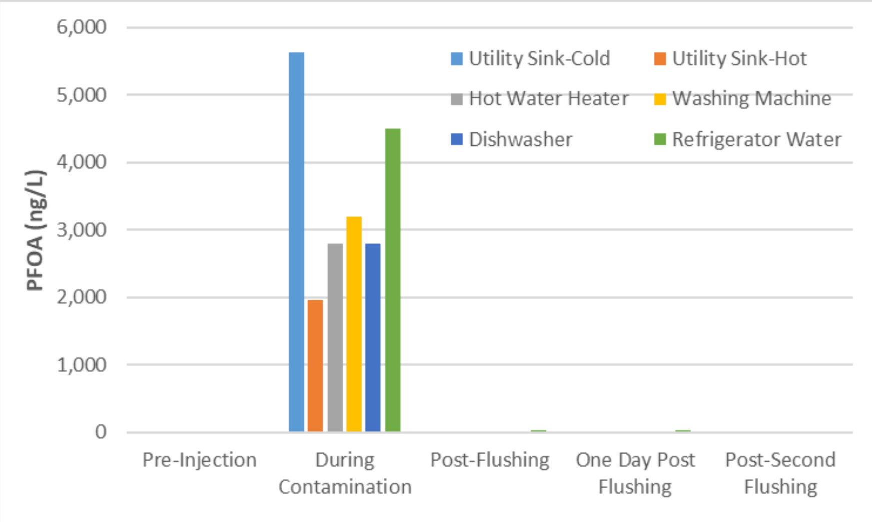


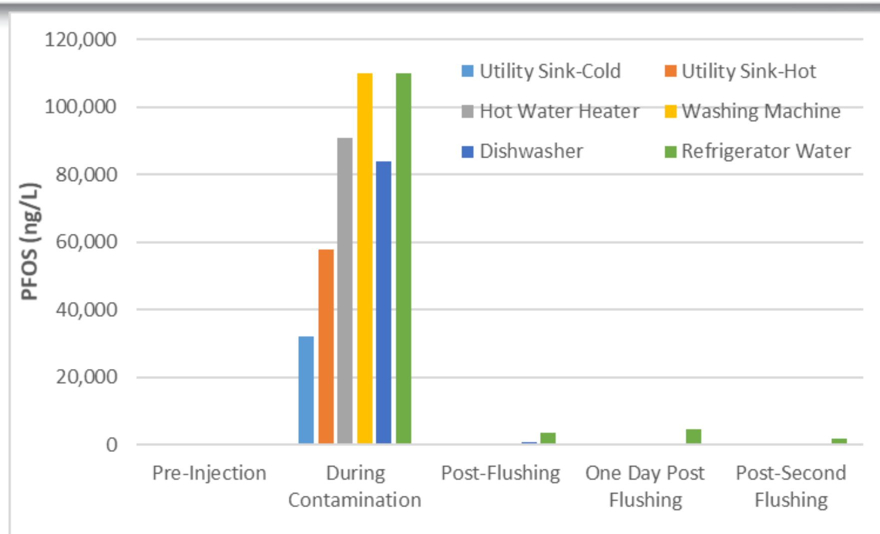


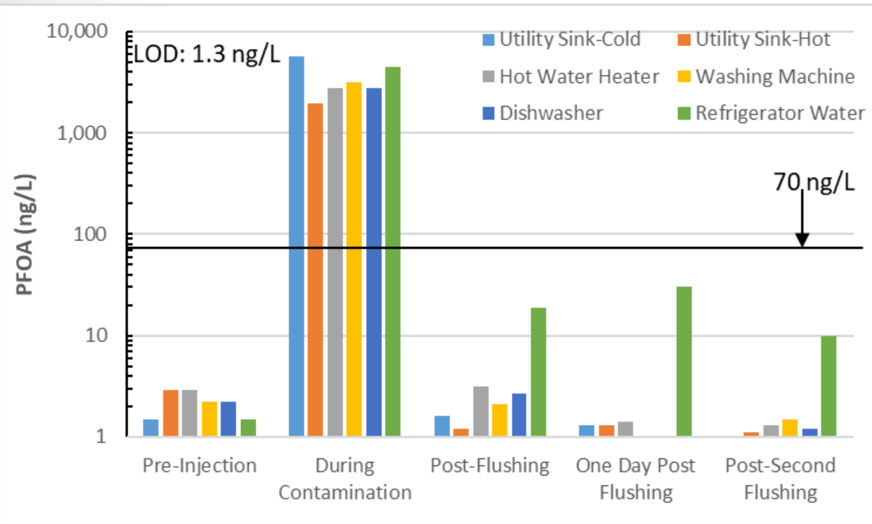


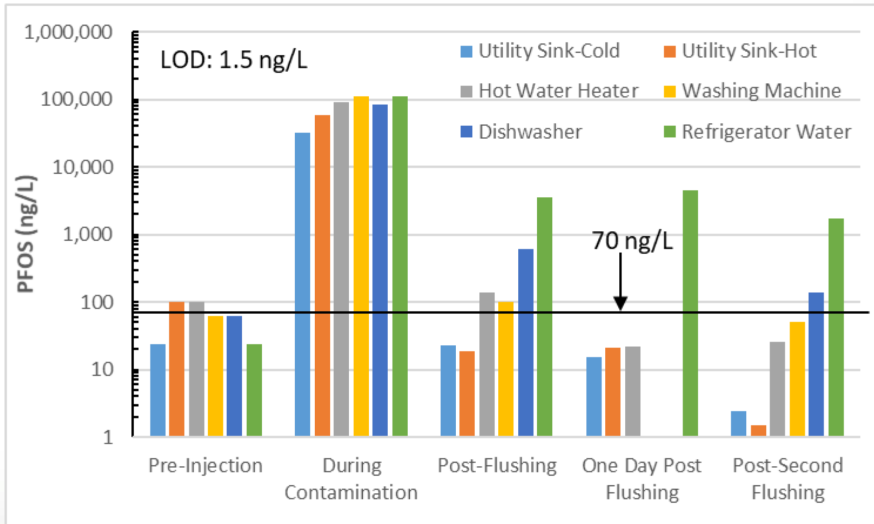


AFFF Flushing Data









Home Plumbing Decontamination Practical Flushing Considerations

- Simple flushing worked better for the washing machine, hot water heater, and utility sink.
- Detergents were not helpful and added additional flush out time.
- If attempting to decon appliances such as refrigerators or dishwashers, consider replacing the hoses and tubing (replacement kits and Youtube videos available).
- Hot water heater:
  - De-energize, disconnect, drain completely through drain valve, and refill with clean supply water separately and before hot and cold water supply lines.
  - Allow time for heating elements to cool before draining.
  - Hot water is supplied to the home hot water lines via a dip tube that draws from around the middle depth of the tank. The bottom 1/3 or so of the tank is dead space for hold up contaminant if the tank is not completely drained.
  - Replace gate valves with globe valve if necessary to allow for more thorough cleaning. Salt/ sediment buildup in the tank may make complete draining impractical and was not studied by these experiments.
- Remember that modern LEED low flow fixtures do not provide the same flow volumes as older or more common fixtures, and may require longer flushing times.

Other Factors: DIY Decon or Replace

- Mechanical skills of the homeowner
- Installed shut off valves for appliances, hot water heater, and fixtures.
- Access to tools and supplies
- Access to floor drains for hot water heater tank decon.
- Insurance coverage
- Cost of appliance and fixture replacement
- Timing and availability of appliance replacements after a widespread event.
- Disposal options for contaminated appliances and fixtures.

WSTB Current and Future Experiments

Accomplished

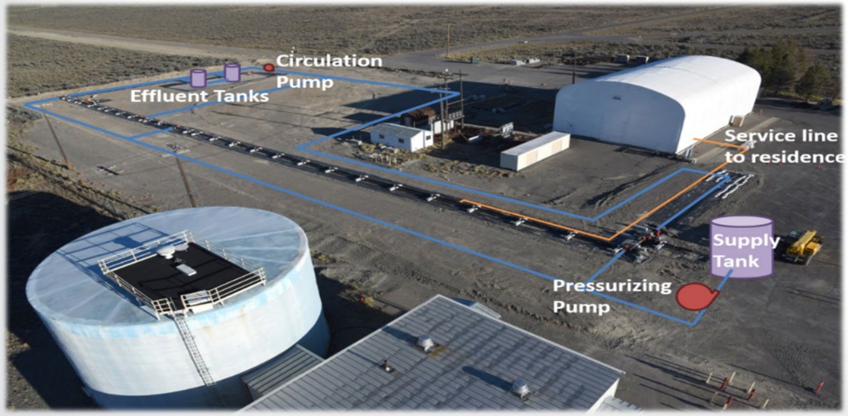
- Persistence of *Bg* spores
- Efficacy of Chlorine dioxide
- Physical scouring of pipes
- **Bakken crude oil flushing**
- **Premise plumbing decon**
- Wash water treatment
- PFAS water treatment

Planned Experiments

- Additional Re-lining Technologies
- Challenge of Mobile Emergency Water Treatment Systems
- Premise Plumbing Low Pressure Contamination Injection
- Detection/Decontamination of radionuclides

SME Recommended Future Opportunities

- Build a larger distribution grid (2 or more city blocks)
- Evaluate other contaminants especially other types of crude oil
- Integrate cyber-security activities

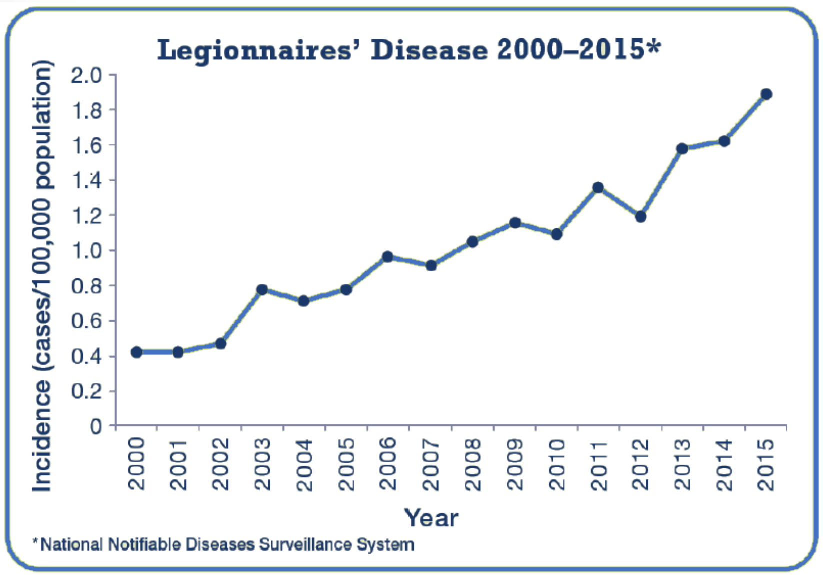


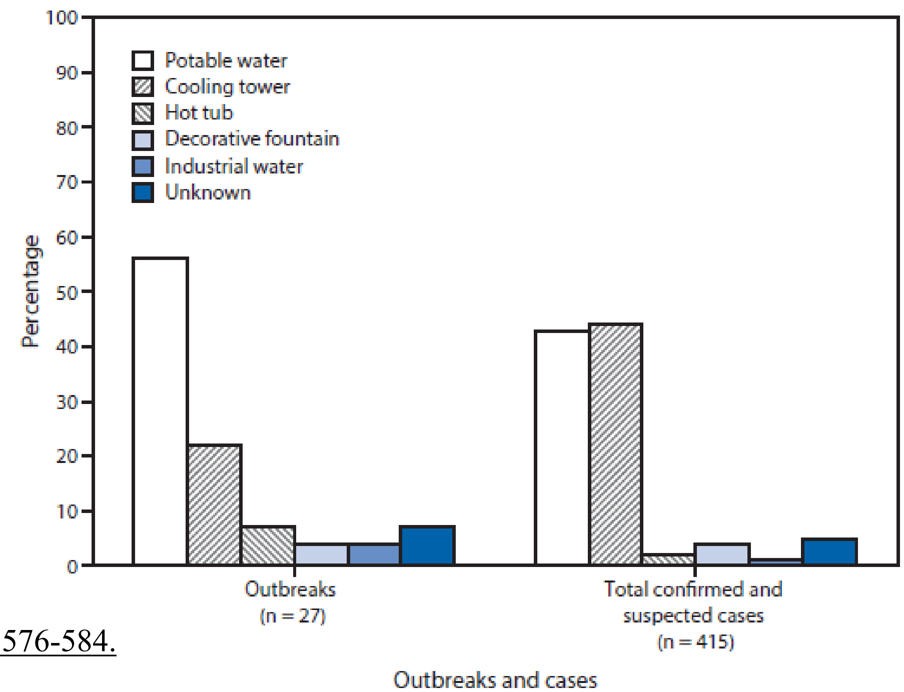
United States Environmental Protection Agency

Disinfection of Biofilm-Associated *Legionella pneumophila*

Office of Research and Development
National Homeland Security Research Center
Water Infrastructure Protection Division

Legionnaires' Disease – A Public Health and Economic Burden



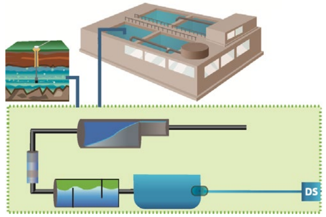


Garrison, L. E., et al. (2016). MMWR Morb Mortal Wkly Rep **65**(22): 576-584.
Collier, S. A., et al. (2012). Epidemiol Infect **140**(11): 2003-2013.

- Incidence rate up from 0.4 to 1.9 cases per 100,000 people; exposure to potable water responsible for the majority of outbreak cases
- Between 2004-2007, LD cases had associated healthcare costs of $9.4 million (Medicaid and Medicare patients)

Legionella Environmental Distribution

Treated water - reclaimed (i.e. sewage treatment plant), ground, and surface

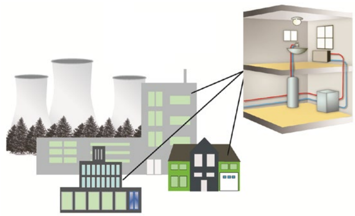


3 x 10^3 – 8 x 10^4 CFU L^{-1}

290 – 2.5 x 10^3 CE L^{-1}

L. anisa
L. adelaidensis
L. bozemanii
L. donaldsonii
L. dumoffi
L. fairfieldensis
L. fallonii
LLAP 2, 3, 4, 7, 8
L. londiniensis
L. micdadei
L. pneumophila
L. quateirensis
L. sterigerwaltii
L. wadsworthii
L. waltersii
L. worsleiensis

Distributed water - premise plumbing, e.g. residences, apartments, recreational facilities, hospitals, and cooling towers



43 – 6 x 10^5 CFU L^{-1}

< 2 x 10^3 – 8 x 10^5 GU L^{-1}

1.6 x 10^5 – 2 x 10^6 CE L^{-1}

L. anisa
L. bozemanii
L. cherrii
L. dumoffi
L. erythra
L. feelii
L. gormanii
L. gresilensis
L. hackeliae
L. longbeacheae
L. lytica
L. maceachernii
L. micdadei
L. pneumophila
L. rubrilucens
L. saintcrucis
L. sterigerwaltii

Soil – garden, compost, potting

10^2 - 10^8 CFU g^{-1}

10^4 - 10^6 GU g^{-1}

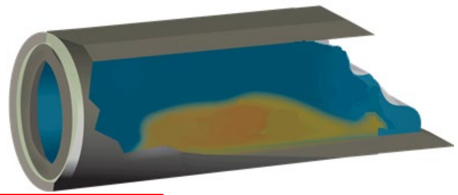
L. anisa
L. birminghamensis
L. bozemanii
L. cincinnatiensis
L. dumoffi
L. feelii
L. gormanii
L. gratiana
L. impletisoli
L. jamestowniensis
L. longbeacheae
L. micdadei
L. nautarum
L. oakridgensis
L. pneumophila
L. quinlivanii
L. saintcrucis
L sainthelensis
L. wadsworthii
L. yabuuchiae

Papadakis et al. 2018. Int J Environ Res Public Health
Schalk et al. 2014. Int J Infect Dis.
van Heijnsbergen et al. 2016. Appl Environ Microbiol.
van Heijnsbergen et al. 2014. J Appl Microbiol.

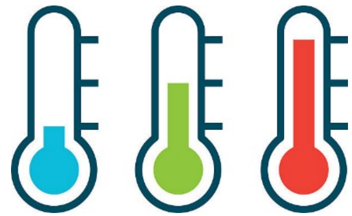
Adapted from Buse et al. 2012. Water Res 46: 921-933.

Legionella's Niche Within Premise Plumbing

generation of respirable, DW-derived aerosols



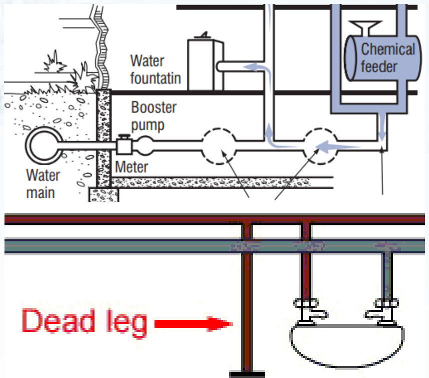
biofilms and sediments



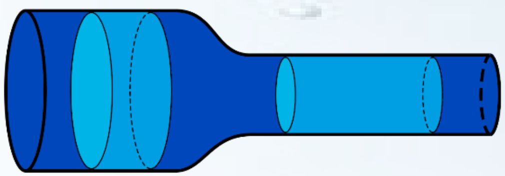
amenable growth temperatures

low to absent residual

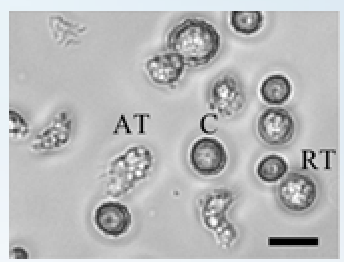
nutrients (metals, minerals, microbes, etc.)



cross-connections



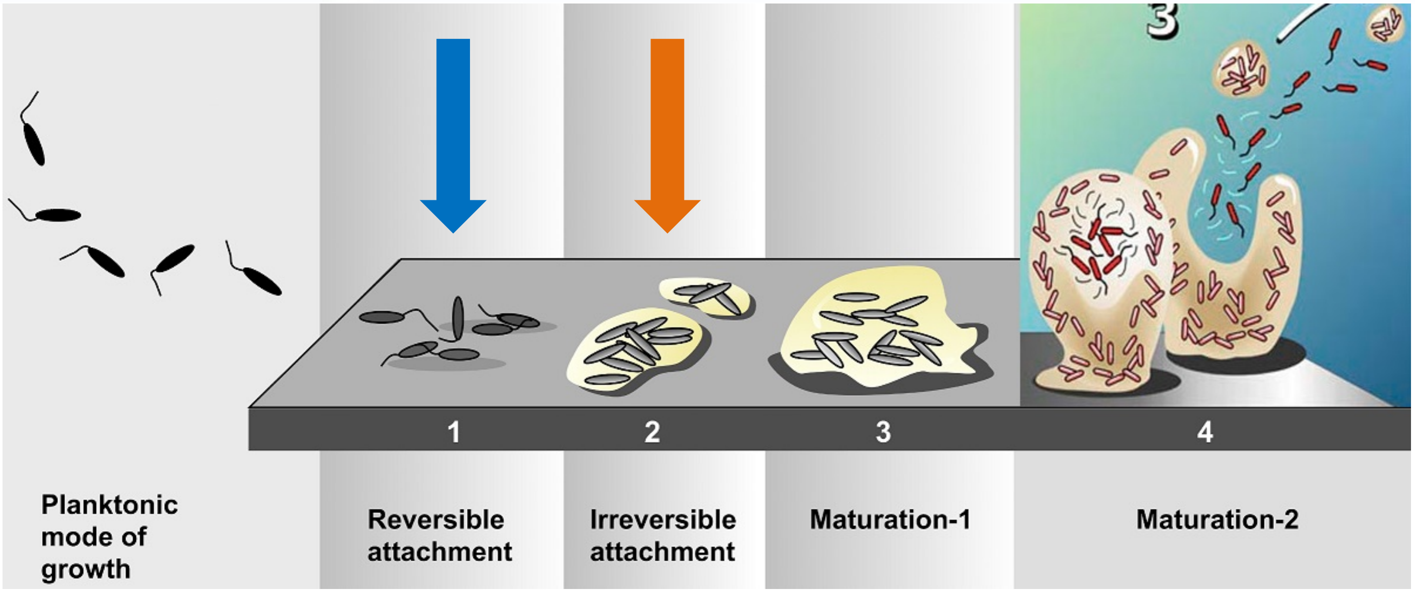
high surface area to volume ratios



eukaryotic hosts

water stagnation

Biofilm Formation

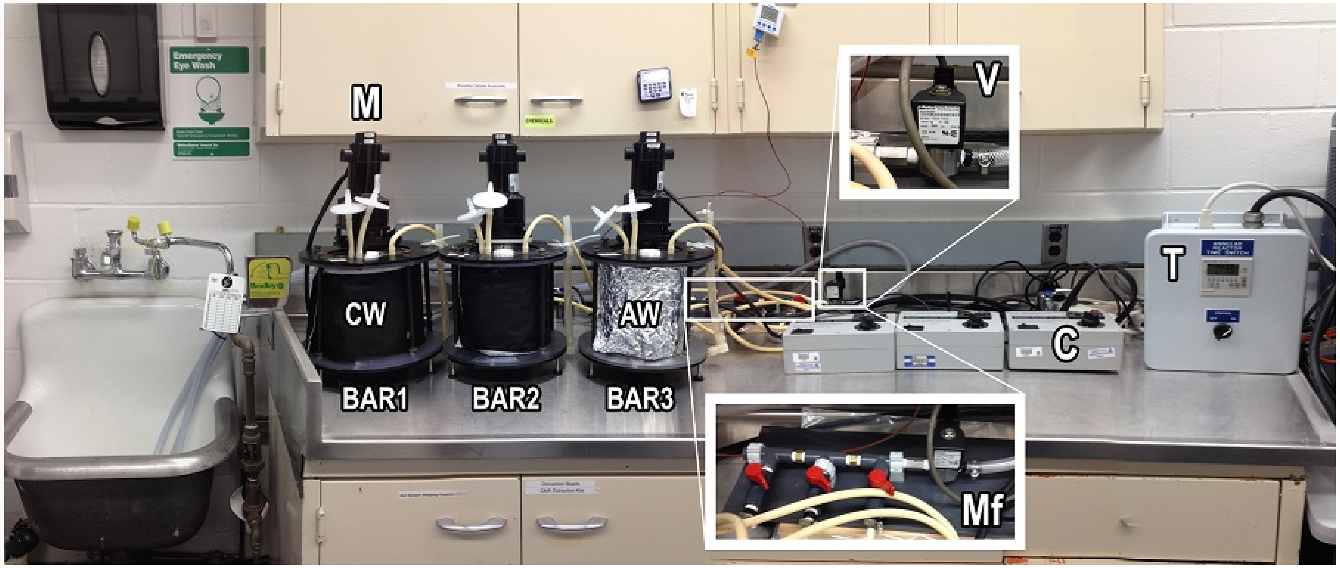


Adapted from Petrova and Sauer. 2009. PLoS Pathogens. doi: 10.1371/journal.ppat.1000668
Adapted from P. Dirckx. 2003. Center for Biofilm Engineering – Montana State University, Bozeman, MT

- Biofilm formation: attachment, proliferation/formation of colonies, and detachment/downstream colonization
- Initial attachment (two stages):
  - Reversible attachment: dependent on physical forces (e.g. electrostatic charge, hydrophobicity, and van der Waals interactions)
  - Irreversible attachment: involves cellular and molecular interactions

Drinking Water Biofilm Development

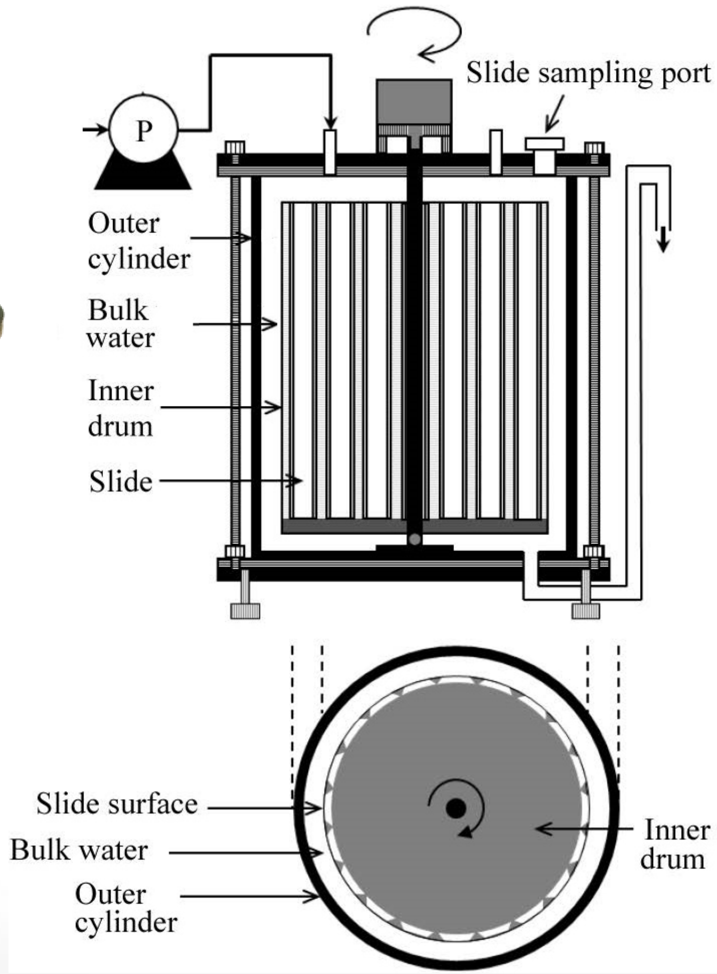
Project goal: determine the disinfection efficacy of chlorine and monochloramine, on DW biofilm-associated *L. pneumophila* established on copper (Cu) and polyvinylchloride (PVC) surfaces



BAR – biofilm annular reactor (1, 2, and 3)
M – motor
CW – cloth wrap
AW – aluminum wrap
Mf – manifold
V – value
C – controller
T – timer

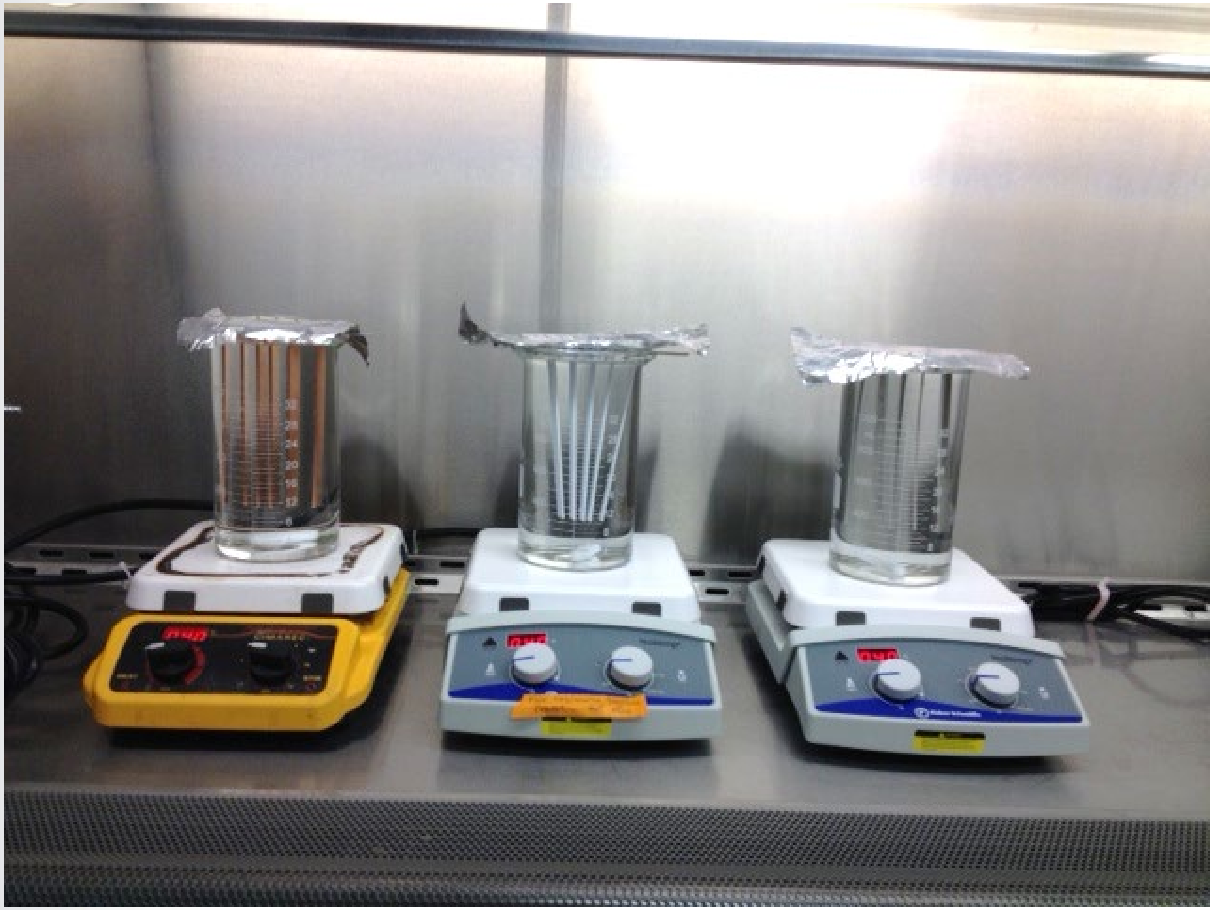
- Developed mature (1.5-2 yo) drinking water biofilms on Cu and PVC surfaces
- Conditions set to mimic private residential water flow conditions
- Monitor water quality: TOC, HPC, chlorine decay, pH, temperature, hardness, turbidity, organics, inorganics, metals, etc.

DW Biofilms on Cu and PVC surfaces



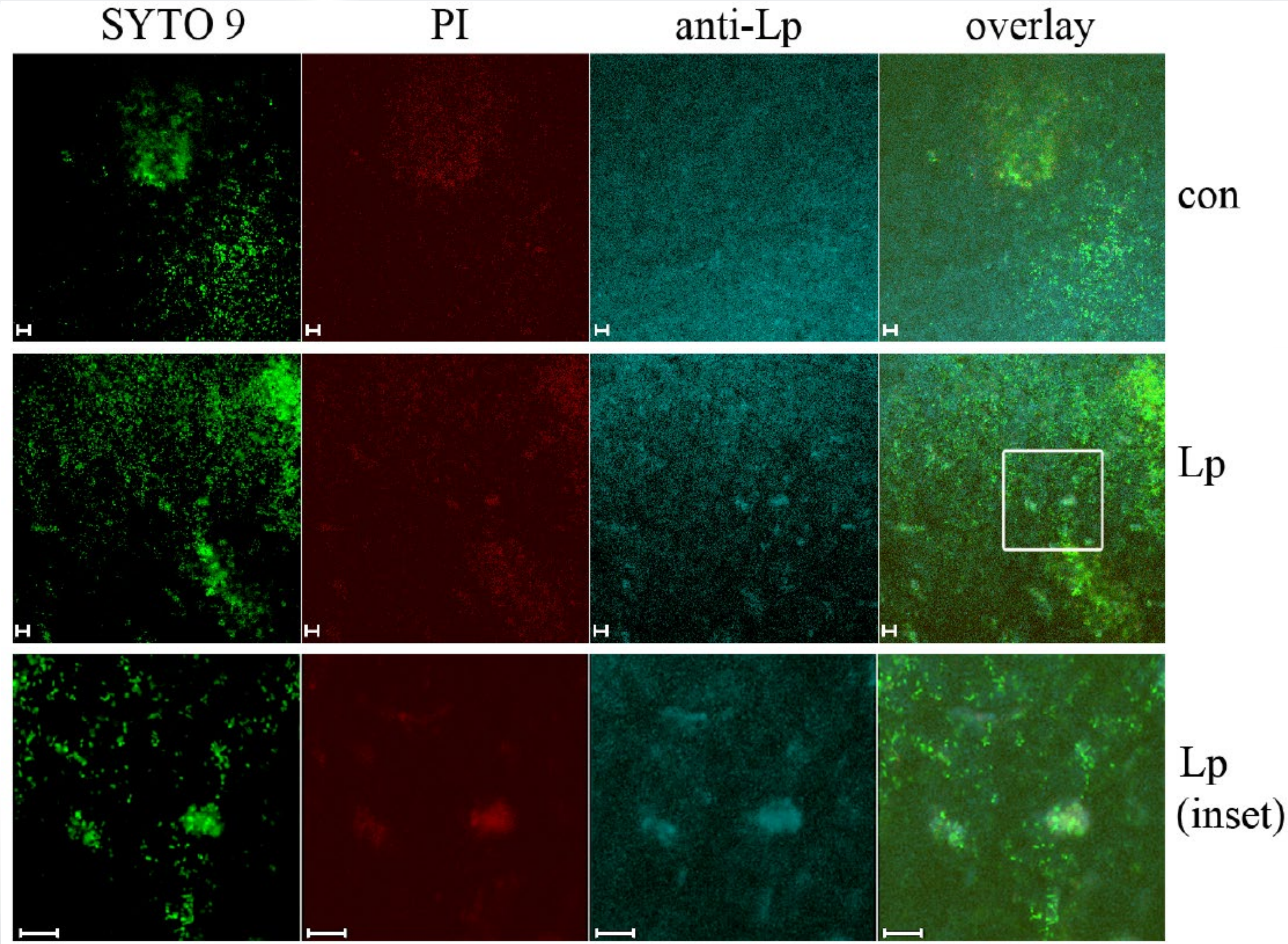
Adapted from Park et al. 2015. K J Microbiol 51:355.

L. pneumophila Inoculation Method

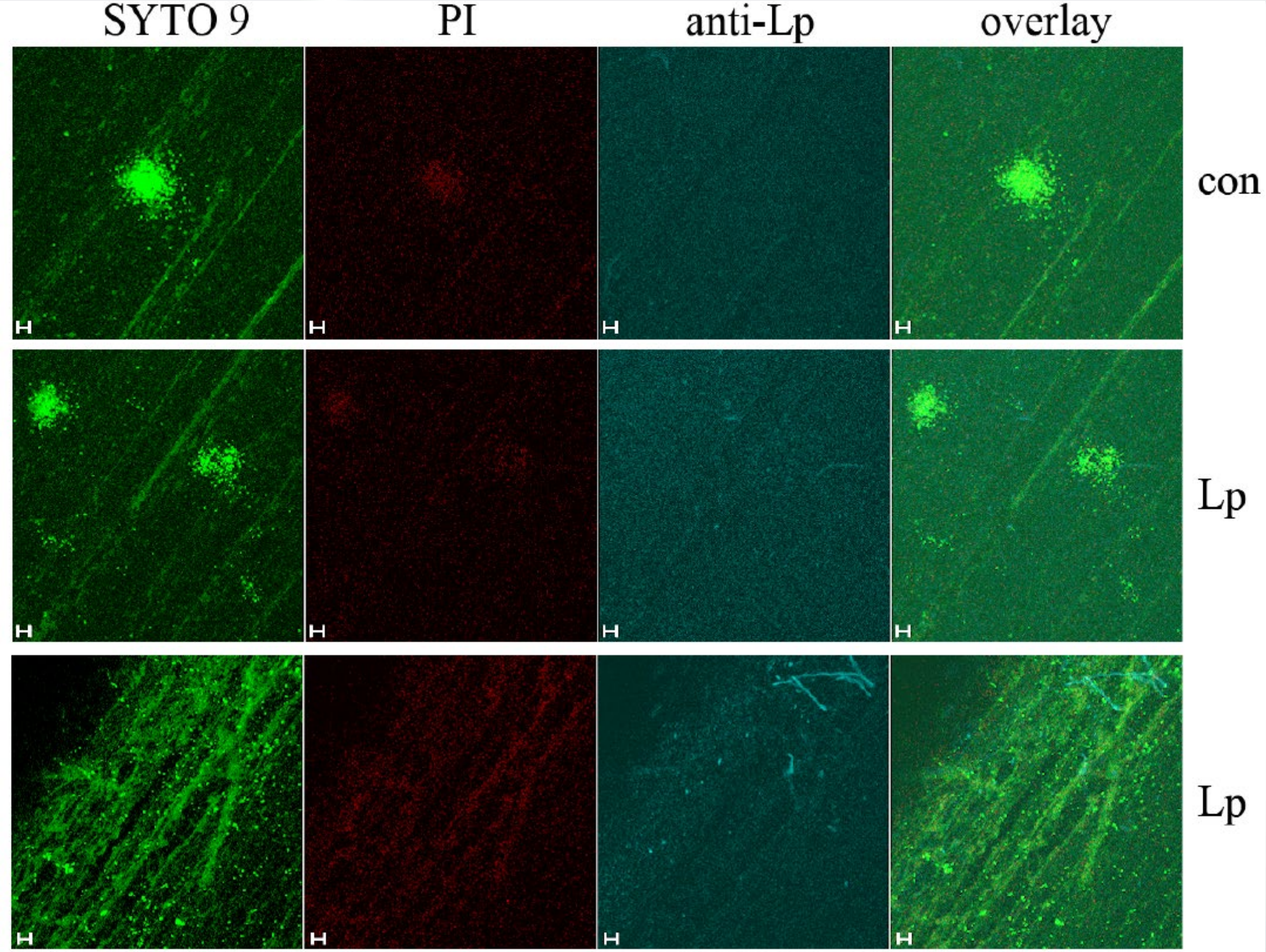


- slides removed from reactors
- suspended in beakers with 10^6 CFU mL^{-1} Lp sg 1 in 0.22 µm filtered, UV dechlorinated (5 d) drinking water (dfH_2O)
- batch mode for 5 d
- slides rinsed twice in 250mL dfH_2O
- incubated for additional 24 h in dfH_2O

Confocal Microscopy – Cu Biofilms



Confocal Microscopy – PVC Biofilms



Disinfectant Inactivation of Microbes

- Concentration x Time (Ct) values =

  [disinfectant concentration, mg L^{-1}] x [contact time, min] = mg min L^{-1}

  – Used for determining inactivation (disinfection) credit during drinking water treatment

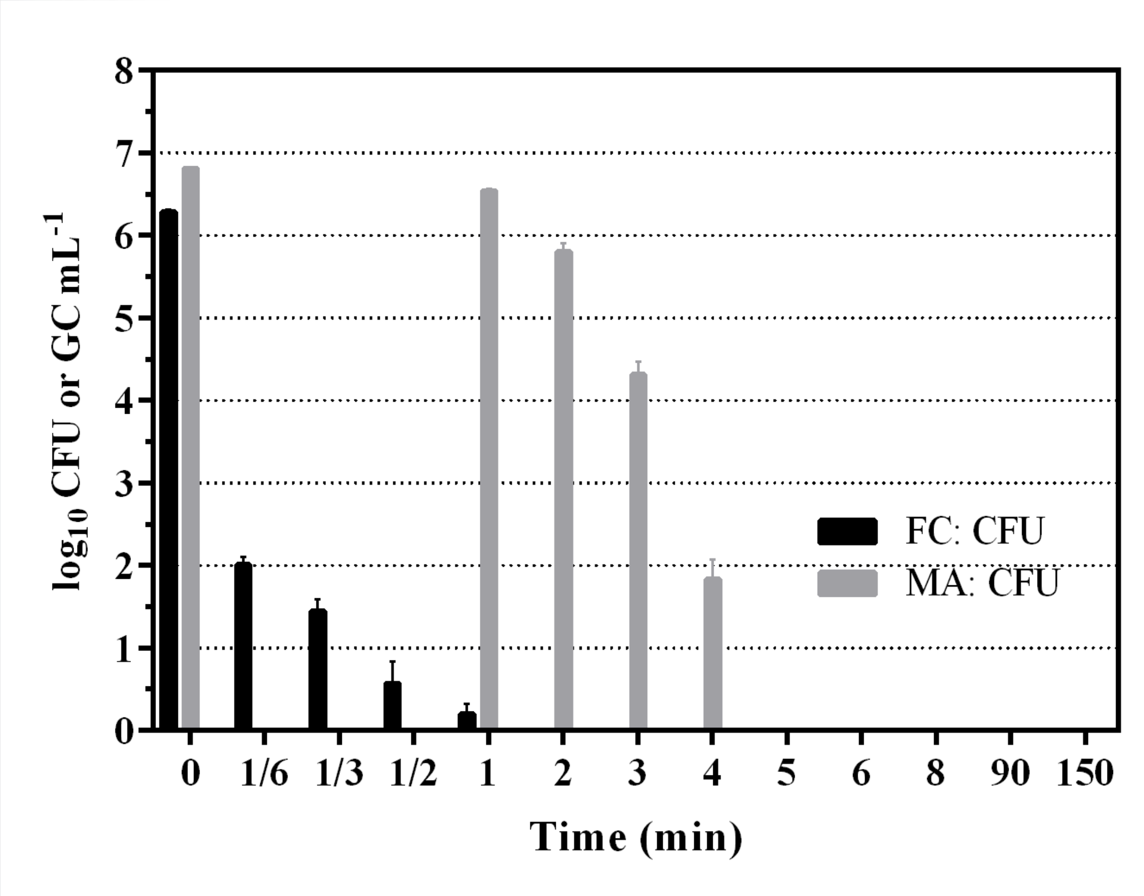
- Log reduction/removal:
  – e.g. SWTR, IESWTR, LT1ESWTR, LT2ESWTR - 99.9% (3-log) removal and/or inactivation of *Giardia lamblia* cysts, 99.99% (4-log) removal of viruses, and 99% (2-log) removal of *Cryptosporidium*

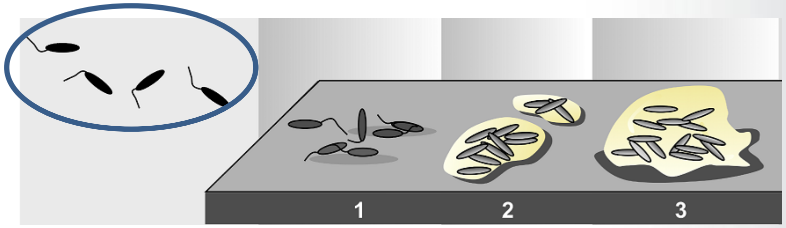
Log Reduction	Reduction percentage
0	0
1	90
2	99
3	99.9
4	99.99
5	99.999
6	99.9999

- Free chlorine and monochloramine inactivation using 2 mg L^{-1}
  – WHO[1]: 5 mg L^{-1} for chlorine and 3 mg L^{-1} for monochloramine
  – ≤ 1.5 mg L^{-1} shown to be inadequate for microbial control[2, 3, 4]
  – Microbial protection from biofilms and disinfectant penetration (50µm):
    - SS biofilms[5]: 2.5 mg L^{-1} FC exposure (30 min); 70% (~1.8mg L^{-1})
    - PC biofilms[6]: 2.6 mg L^{-1} FC and MA exposure: 8% FC (0.2 mg L^{-1}) and 15% MA (0.4 mg L^{-1})

[1]WHO. 2017. Guidelines for Drinking-water Quality, 4th Edition.
[2]LeChevallier et al. 1987. Appl Environ Microbiol 53: 2714-24.
[3]Neden et al. 1992. J AWWA 84: 80-88.
[4]Wolfe et al. 1990. Appl Environ Microbiol 56: 451-462.
[5]De Beer et al. 1994. Appl Environ Microbiol 60: 4339-44.
[46]Lee et al. 2011. Environ Sci Technol 45:1412-19.

Inactivation of Planktonic *L. pneumophila*

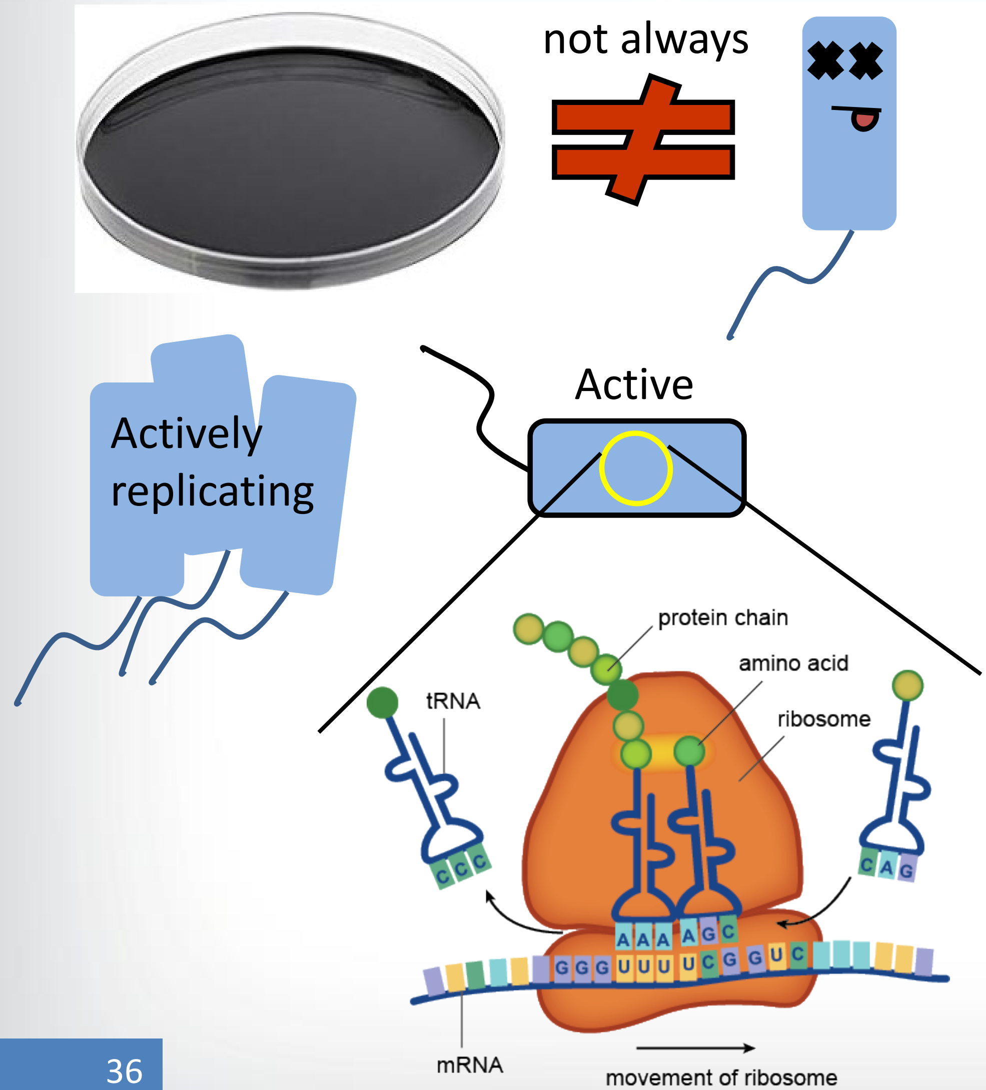


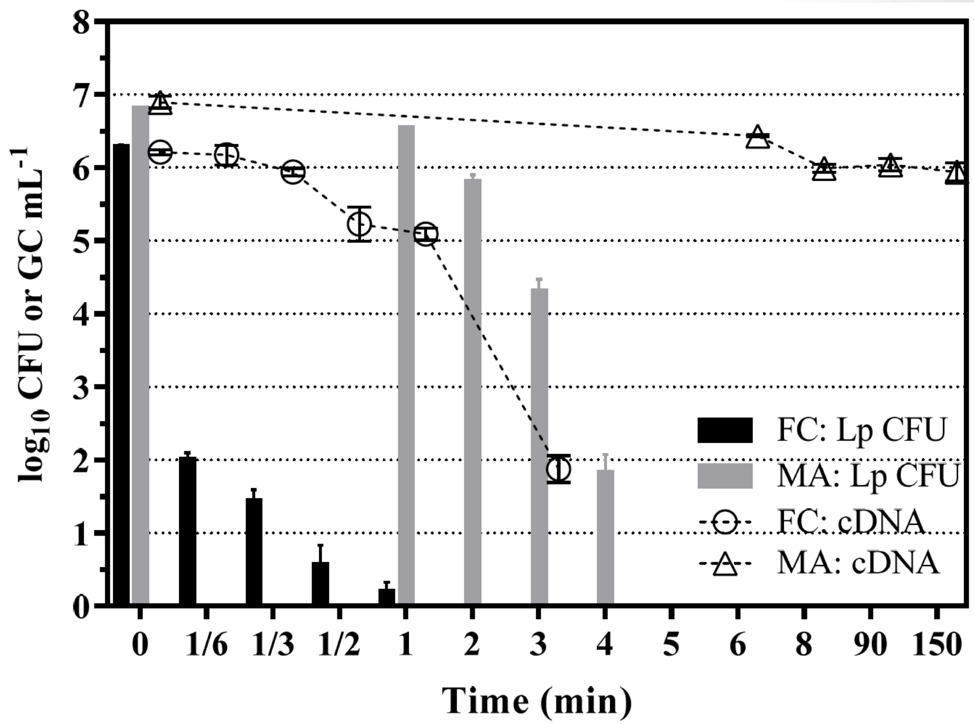


mg min L^{-1}

Inactivation Ct value for:	FC	MA
2-$\log_{10}$	-	5.35
3-$\log_{10}$	0.11	6.58
4-$\log_{10}$	0.30	7.81

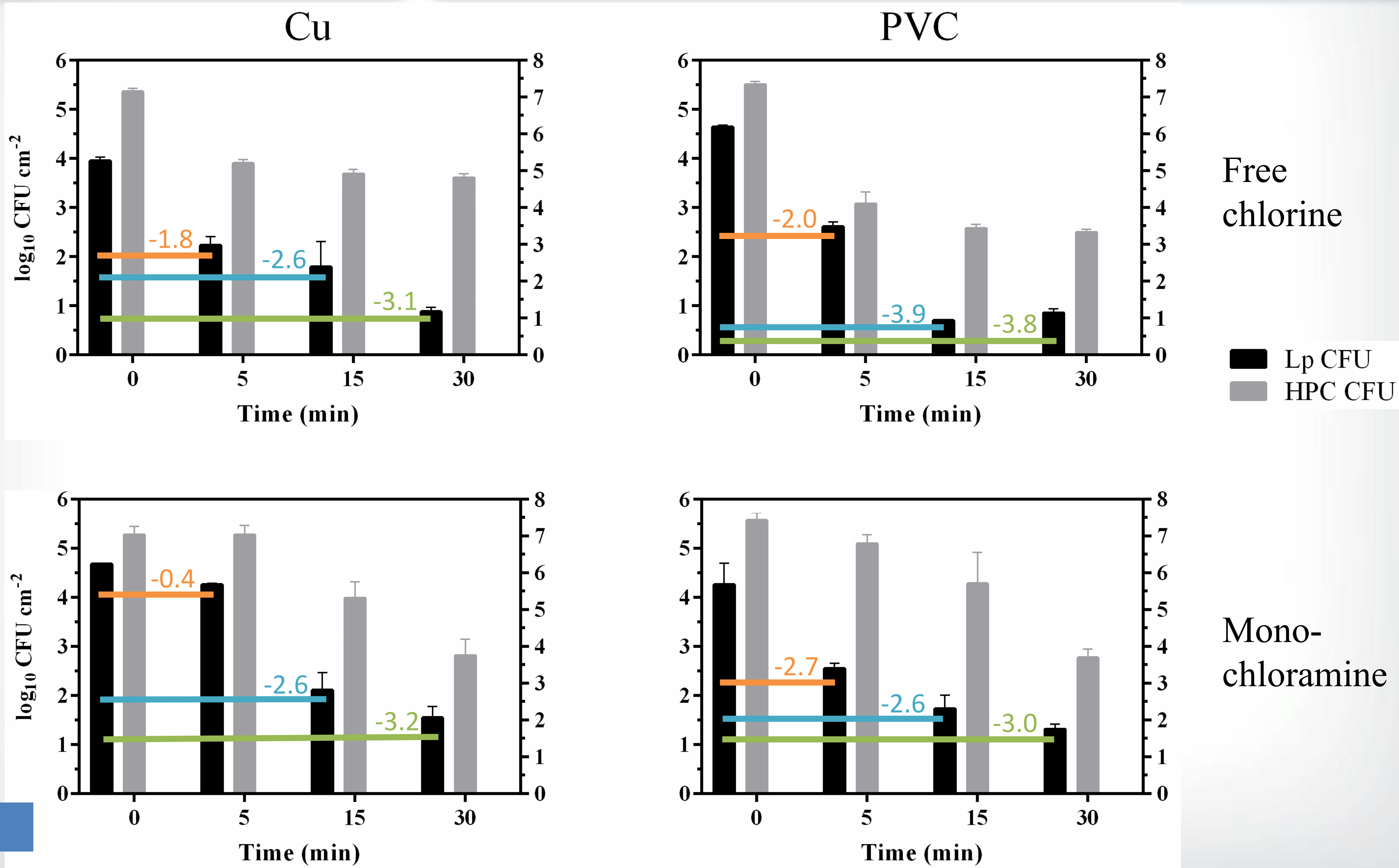
Inactivation of Planktonic *L. pneumophila*





- Total RNA → cDNA → Lp 16S rRNA qPCR
- Levels declined rapidly, ~3min for FC
- Levels NS different, up to 2.5 hours for MA
- Most likely due to FC being highly oxidative

Inactivation of Lp-Colonized Cu and PVC DW Biofilms

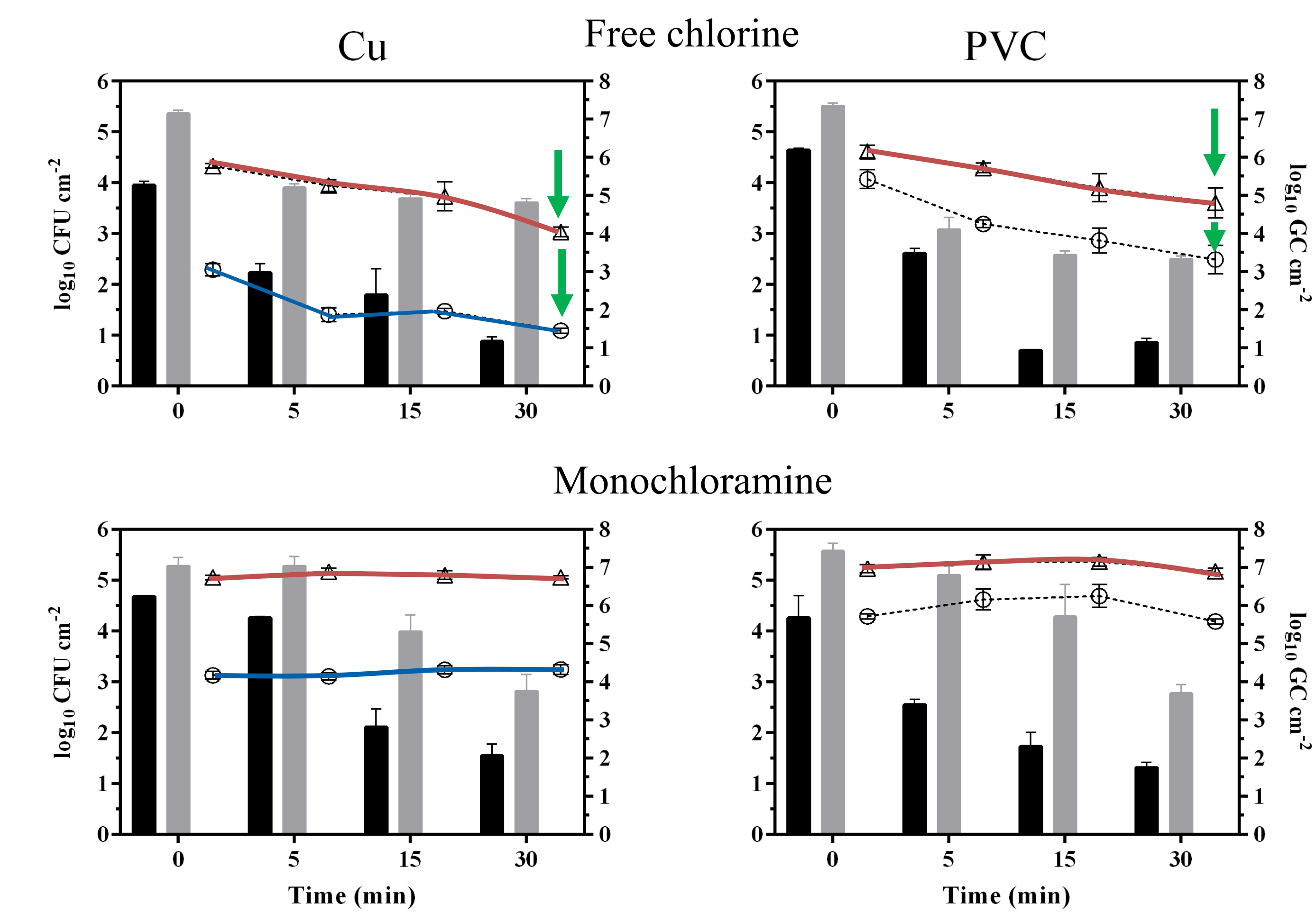


Ct values for Planktonic- and Biofilm-Lp inactivation

Inactivation *Ct* value for:	Planktonic Lp		Drinking Water Biofilm-associated Lp			
	Free chlorine	Monochloramine	←Lower *Ct*			Higher *Ct*→
2-log_{10}	-	5.35	Free chlorine PVC 8.86	Free chlorine Cu 13.18	Monochloramine PVC 17.16	Monochloramine Cu 34.86
3-log_{10}	0.11	6.58	Free chlorine PVC 36.11	Free chlorine Cu 50.83	Monochloramine Cu 55.38	Monochloramine PVC 62.8
4-log_{10}	0.30	7.81	Free chlorine PVC 63.67	Monochloramine Cu 75.90	Free chlorine Cu 88.48	Monochloramine PVC 108.44

- 2- and 3-log reduction biofilm-Lp: free chlorine more effective at inactivation
- For 4-log reduction: Effectiveness seemed to be substratum and disinfectant dependent
  - Biofilm penetration? (Cu vs. PVC biofilm structures)
  - Chemical interactions between disinfectant and substratum?

16S rRNA Gene and Transcript Levels During Inactivation



Lp 16S rRNA gene:

- Δ: total DNA levels
- o: transcript levels (total RNA → cDNA)

- gene levels from total DNA were higher than transcript levels

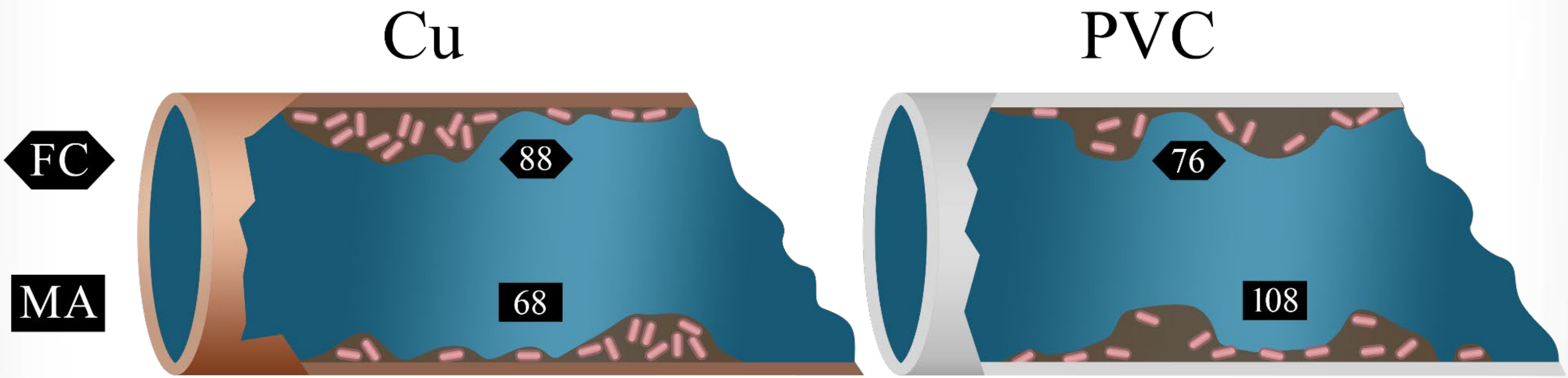
- Free chlorine treatment of biofilm-Lp statistically impacted gene and transcript levels

- Transcript levels lower in Cu biofilms compared to PVC biofilms (t = 0 min, similar CFU levels)

Summary Points

- For planktonic *L. pneumophila*, free chlorine (FC) was more effective at inactivation compared to monochloramine (MA) treatment

- For biofilm-associated *L. pneumophila* and 4-log inactivation levels,
  MA was more effective on Cu biofilms, while FC was more effective on PVC biofilms



- Free chlorine treatment negatively impacted 16S rRNA gene transcript levels and may act synergistically with Cu surfaces to further reduce their levels

Acknowledgements

Biofilm work

US EPA

Jeff Szabo* PhD, NHSRC

David Wahman, PhD, NRMRL

Aptim/PTSI

Brian Morris*

Ian Struewing*

University of Cincinnati

Live Microscopy Core

Chet Closson

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WSTB

Idaho National Lab

Stephen Reese

Michael Carpenter

Travis McLing

Aptim

Greg Meiners

Sue Witt

Sri Panguluri

THANK YOU